

Claims

## WHAT IS CLAIMED IS:

- sub B<sup>1</sup> 1. A method for producing a recombinant protein in an insect larvae expression system, the method comprising:
- (a) infection of larvae with a vector that has a nucleic acid sequence that encodes a recombinant fusion protein with an affinity tag wherein the recombinant protein is expressed in the larvae; and
  - (b) purification of the recombinant protein from said larvae by affinity chromatography.
2. The method of claim 1 wherein the recombinant fusion protein is a membrane fusion protein.
3. The method of claim 1 wherein the affinity tag is selected from the group consisting of poly(His), avidin, biotin, antibody, streptavidin and an antigenic amino acid sequence.
4. The method of claim 3 wherein the affinity tag is poly(His).
5. The method of claim 1 wherein the vector is a baculovirus.
6. The method of claim 1 wherein the larvae are infected with the vector when the larvae are in the first, second, third, or fourth instar stage of development.
7. The method of claim 1 wherein the larvae are in the early fourth instar stage of development.
- sub B<sup>2</sup> 8. ~~The method of claim 1 further comprising isolation of a protein fraction.~~

from the larvae wherein the ~~fraction contains the recombinant fusion protein with the~~  
~~affinity tag.~~

Sub C2 → 9. The method of claim 8 wherein the fraction is isolated from the larvae by differential and gradient centrifugation.

10. The method of claim 9 further comprising isolation of the fraction by chromatography performed after the step of differential and gradient centrifugation.

Sub B<sup>3</sup> → 11. The method of claim 1 further comprising removal of the affinity tag from the recombinant fusion protein.

12. The method of claim 2 wherein the recombinant membrane fusion protein is selected from the class of proteins consisting of transport, channel forming, receptor, junctional, cytoskeletal, and other membrane associated proteins.

Sub C2 → 13. The method of claim 12 wherein the recombinant membrane protein is a transport protein.

14. The method of claim 13 wherein the transport protein is NCX1 or the Na-K ATPase.

Sub C2 → 15. The method of claim 12 wherein the recombinant membrane protein is a channel forming protein.

16. The method of claim 15 wherein the channel forming protein is CFTR.

Sub C2 → 17. The method of claim 12 wherein the recombinant membrane protein is a junctional protein.

18. The method of claim 17 wherein the junctional protein is connexin 32.

sub B4

- ~~19. The method of claim 1 wherein the recombinant fusion protein has biological activity substantially the same as the native form of the protein.~~

20. The method of claim 1 wherein the recombinant fusion protein has substantially the same structure as the native form of the protein.

Sub C2 fusion

21. A method for identifying the physical characteristics of a recombinant fusion protein wherein the protein is produced by the method of claim 1.

22. The method of claim 21 wherein the physical characteristics are determined by a procedure selected from the group consisting of crystallography, NMR, and CD

23. The method of claim 22 wherein the procedure is crystallography.

add  $B^4$